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T E C H N I C A L R E P O R T

TO

OFFICE OF NAVAL RESEARCH
UNITED STATES DEPARTMENT OF THE NAVY

BIOLOGICAL SCIENCES DIVISION
CLINICAL BRANCH
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"PLANT COLLOIDS FOR EVALUATION AS PLASMA EXTENDERS AND FOR COMPLEXING WITH PROTEIN SPARING SUBSTANCES"

REPORT NO. TEN

URS F. NAGER

MARCH 1, 1953

Synthetic Blood Plasma Extenders

Introduction

This report covers work during February on glycerol pectate for use as plasma extender. Special attention in view of increasing the yields was given to the synthesis of glycidol, the esterifying agent used in preparing this pectate derivative. Additional amounts of glycerol pectate were made for evaluation on animals.

Summary

The yields on glycidol prepared by reacting glycerol α-monochlorohydrin with caustic were shown to vary with the purity of the starting material. Losses due to polymerization were particularly high for less pure glycerol α-monochlorohydrin and appeared to be caused by traces of alkali accumulating in isolating the glycidol from the reaction mixture. The yields were markedly improved when concentration and distillation of the glycidol solution was carried out at the lowest possible temperature and in a CO_2 atmosphere. Under these conditions, polymerization was considerably retarded.

The preparation of glycerol pectate was continued for animal studies conducted by Dr. F. W. Hartman of Henry Ford Hospital Research Laboratories. A larger run was made with commercial Pectic Acid #75 and purified glycidol. The glycerol pectate was reprecipitated to eliminate possibly traces of adherent polyglycerol and was simultaneously fractionated. Two batches of equal weight were obtained. Although their oncotic pressures were essentially alike, they showed differences in relative viscosity.

Additional leads to the mechanism of reaction between sodium pectate and glycerol α-monochlorohydrin explained the failure to yield glycerol pectate. A different route was then investigated by reacting pectic acid with glycerol α-monochlorohydrin in presence of a HCl acceptor such as a basic ion-exchange resin. Evidence for partial esterification by this method was demonstrated with Amberlite IRA-410.

The author has consulted and collaborated with Dr. Rene G. Jennen on various problems relative to this project.

Experimental

1. Preparing Glycidol

Considerable time was devoted to improving the yields of glycidol as prepared by reacting glycerol α-monochlorohydrin with caustic. Earlier experiments indicated that spontaneous polymerization was one of the main reasons responsible for the low yields on glycidol. Working with glycerol alpha-monochlorohydrin obtained from three different manufacturers, it was further shown that the yields of glycidol varied considerably and appeared to have some relationship to the purity of the starting material.

The polymerization of glycidol is known to be catalyzed by strong acid or alkali. When glycerol α-monochlorohydrin and caustic were reacted in stoichiometric amounts, the reaction mixture showed slightly alkaline reaction (pH 8-9). The use of less than the theoretical amount of caustic did not influence the pH of the reaction mixture. Whether this slightly alkaline reaction was due to sodium-carbonate introduced with the caustic or whether it was due to hydrolysis of the sodium salt of an organic acid present in the starting material remains to be demonstrated. As the reaction mixture was evaporated to remove isopropanol and water and finally distilled, the alkali may have then accumulated in sufficient concentration to induce polymerization.

It was therefore attempted to control the pH by introducing CO_2 during the distillation. The experiments summarized below were all made with a charge consisting of 8 moles glycerol α-monochlorohydrin, 40 moles isopropanol and 8 moles of NaOH. The chlorohydrin was diluted with the isopropanol and chilled to $-5^{\circ}C$. Over a period of 1 to 1-1/2 hours, the caustic was slowly added as a 50% aqueous solution. Agitation was continued for two more hours. The NaCl was removed by filtration, rinsed with isopropanol and dried. The filtrate, to which was added a small amount of dry ice, was then vacuum concentrated at $<40^{\circ}C$. by passing in CO_2 through a capillary. On the following day the glycidol concentrate was distilled at 5-6 mm Hg pressure. The following table summarizes the results obtained on four consecutive runs.

Run No.	NaCl Recovered g	NaCl Recovered %	Glycidol Yield g	Glycidol Yield %
P-146	435	93	115	20
P-150	440	94	347	60
P-154	415	89	335	58
P-155	435	93	362	63

The data show that the conversion of glycerol α-monochlorohydrin with caustic was nearly quantitative as the amount of NaCl isolated by filtration of the reaction mixture was above 90%, not including an additional crop of NaCl crystallizing during concentration and evaporation. In run P-154, the caustic solution was filtered to remove carbonate. The slightly lower conversion may therefore be explained by mechanical losses of caustic.

The above results further indicate a striking difference in the yields of glycidol, an increase from 20 to 60% using CO_2 to control the pH. The distillation residues also differed markedly. Run P-146 yielded an extremely viscous oil which was apparently a high molecular weight polyglycerol designated Lot #P-146. The distillation residues of the subsequent runs made in presence of CO_2 gave light mobile oils which were apparently lower molecular weight polymers. When treated with a trace of strong caustic they continued to polymerize yielding material similar to Lot #P-146.

Further improvements of yields on glycidol appear to depend on complete removal of alkali from the reaction mixture and rapid distillation of the concentrate to avoid prolonged exposure to elevated temperatures. The latter has not yet been possible with the available distillation equipment.

Studies are under way to deionize the reaction mixture after filtering off the precipitated salt. It may then be possible to simply remove isopropanol by vacuum distillation and to use the concentrate directly without further distillation.

2. Preparing Glycerol Pectate Lot Nos. 2 and 3

The following batches of glycerol pectate were prepared to provide additional material for evaluation in vivo.

Three 32 ounce beverage bottles were charged, each with 75 grams Pectic Acid #75, 340 ml H_2O and 150 grams glycidol, sealed and tumbled end-over-end in a bath maintained at 35°C. After 15-1/2 hours samples were taken which revealed still slightly acid reaction. At the end of 23 hours the reaction mixture reached neutrality. The contents of the three bottles were combined and bleached overnight with 750 ml saturated ClO_2 solution. The bleached reaction mixture was diluted with water to 3200 ml and filtered with the aid of 1% Dicalite Special Speedflow. Glycerol pectate was precipitated from the filtrate (4100 ml including rinse) by adding an equal volume of acetone. The supernatant liquid was decanted and the solids were rinsed by slurrying and decanting twice with one liter 75% acetone.

The rinsed glycerol pectate was then redissolved in 3800 ml H_2O and reprecipitated with 4000 ml acetone. Precipitation was obviously incomplete as evidenced by a very milky supernatent. Addition of another liter of acetone was without effect on the emulsion. The precipitate was filtered off and the filtrate was treated separately as described later on.

The solids collected on the filter were rinsed and dehydrated as usual by reslurrying and decanting with several small portions of acetone. After drying for 1-1/2 hours at 70° followed by further drying in vacuo over KOH at 25° for six hours, 96 grams of glycerol pectate Lot #2 were obtained.

The cloudy filtrate mentioned above (about 3 gallons including acetone rinses) was kept at 25° for two days without separating out additional solids. Precipitation was finally achieved by stirring in 180 ml saturated NaCl solution. The supernatant liquid, which now was completely clear, was decanted off. The white, flocculent precipitate was treated by slurrying twice with 500 ml 75% acetone and dehydrated with acetone. After drying under conditions indentical to those described for Lot #2, 96 grams of glycerol pectate, Lot #3, were obtained. The overall yield on glycerol pectate isolated in this run amounted to 85% by weight, based upon pectic acid. Following are the viscosities and oncotic pressures determined on glycerol pectate Lots No. 2 and 3.

	η rel.		Pressure mm H_2O**			
Lot No.	C=4%	C=2%*	24	48	72	144 hrs.
2	9.24	3.25	105	165	200	308
3	6.09	2.55	95	158	204	312

* In saline.
** C=2% in saline against H_2O.

3. Attempted Preparation of Glycerol Pectate by Other Routes

In the previous report experiments were described in the course of which it was attempted to prepare glycerol pectate by reacting sodium pectate with glycerol α-monochlorohydrin. Apparent reasons for the failure of those studies were pointed out. Of particular interest to our work were the results of the reaction between silver stearate and glycerol α-mono--iodohydrin reported in the literature. The authors found that the precipitation of silver iodide was complete before any

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detectable amounts of ester were formed. Instead, they were able to isolate glycidol in high yields by vacuum distillation of the reaction mixture. It was their conclusion that glycidol was formed in primary reaction and that the reaction between glycidol and stearic acid proceeded at a much slower rate.

As a result of the esterification studies made during the past two months, an additional possibility of obtaining glycerol pectate was considered, namely the reaction between pectic acid and glycerol α-monochlorohydrin in presence of a hydrochloric acid acceptor. It was anticipated that an insoluble basic compound such as an anion-exchange resin may convert glycerol α-monochlorohydrin to glycidol by combining with the HCl which is split off. In secondary reaction, the glycidol would then be expected to yield glycerol pectate.

Preliminary attempts along this line are described in the following. Two commercial ion-exchange resins were studied, Amberlite IR-45 (weak base) and Amberlite IRA-410 (strong base). These resins are relatively non-porous and thus adsorb only low molecular weight acids. The individual runs were made in 8 ounce beverage bottles which were tumbled end-over-end in a bath kept at 40°C.

Amberlite IR-45

Run No.	Pectic Acid g	IR-45 g	Chlorohydrin g	H_2O ml
144-1	5	10	3	30
144-2	5	30	10	30

Whereas run #144-1 showed no signs of esterification, a gradual increase in viscosity of the reaction mixture #144-2 was observed. At the end of 72 hours there was still undissolved pectic acid present.

Amberlite IRA-410

Run No.	Pectic Acid g	IRA-410 g	Chlorohydrin g	H_2O ml
141-27	4	20	15	18
144-3	5	20	10	25
144-4	5	40	10	30

After tumbling the bottles overnight at 40°C. highly viscous reaction mixtures resulted yielding weakly acid solutions upon dilution with water. Only a light precipitate was obtained by adding calcium ions. These results were unchanged at the end of 72 hours of incubation.

In summary, the experiments with the strongly basic ion-exchange resin showed that partial esterification took place. Whether it will be possible to achieve complete esterification by this method remains to be demonstrated.